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NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

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in its capacity as elected Office

Date of mailing (day/month/year) 29 October 1999 (29.10.99)	
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International filing date (day/month/year) 24 February 1999 (24.02.99)	**Priority date** (day/month/year) 25 February 1998 (25.02.98)
Applicant BARBUCCI, Rolando et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

21 September 1999 (21.09.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
1845PTWO.		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 99/01191	24/02/1999	25/02/1998
Applicant		
FIDIA ADVANCED BIOPOLYMERS S.R.L.et.al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[ ] the text is approved as submitted by the applicant.

[X] the text has been established by this Authority to read as follows:

SULPHATED HYALURONIC ACID AND SULPHATED DERIVATIVES THEREOF COVALENTLY BOUND TO POLYURETHANES, AND THE PROCESS FOR THEIR PREPARATION

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

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(54) Title: SULPHATED HYALURONIC ACID AND SULPHATED DERIVATIVES THEREOF COVALENTLY BOUND TO POLYURETHANES, AND THE PROCESS FOR THEIR PREPARATION

(57) Abstract

The present invention is directed to novel compounds with a high degree of haemocompatibility constituted by a polyurethane covalently bound to sulphated hyaluronic acid or to its sulphated derivatives, suitable for the preparation of biomaterials and for the coating of biomedical objects in the field of health care and surgery.

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SULPHATED HYALURONIC ACID AND SULPHATED DERIVATIVES THEREOF COVALENTLY BOUND TO POLYURETHANES, AND THE PROCESS FOR THEIR PREPARATION

Field of the invention

The present invention concerns a polyurethane covalently bound to sulphated hyaluronic acid or to its sulphated derivatives, the process for their preparation, and the haemocompatible materials comprising said polyurethane.

State of the art

Considerable efforts have been made over the last few decades in the synthesis and surface modification of constantly new classes of polymers, in order to provide haemocompatible materials for the use in surgery.

Polyurethanes are widely used in biomedical applications because of their good mechanical and haemocompatible properties.

In order to enhance the latter property, molecules able to inhibit the coagulative process have been bound to the surface of polyurethane.

These substances are usually chosen from among those which can prevent platelet adhesion and aggregation, or block coagulation factors.

Heparin is one of the modifying agents used, and it can be bound to the polymer surface by both ionic bonds (US 4,944,767) and covalent bonds (W. Marconi et al., *Makromol. Chem.* 194, 1347-1356, 1993).

These bonds can be achieved once the polymer surface has been chemically modified by introducing reactive groups such as carboxy, hydroxy and amino groups.

However, one of the main drawbacks to the use of heparin is its high degradation rate on account of the enzyme heparinase, which limits its possible applications in fields of surgery such as cardiovascular surgery, which may call for the implant of devices where the absence of thrombogenicity must be guaranteed for lengthy periods.

Other modifying agents with anticoagulant properties are O-sulphated hyaluronic acid and its O-sulphated derivatives, prepared according to the method described in the international patent application by the Applicant, No. WO 95/25751.

Also of considerable importance are N-sulphated hyaluronic acid and its N-sulphated derivatives, optionally salified, wherein the glucosamines are partially N-sulphated or partially N-sulphated and partially or totally O-sulphated in position 6, as described in the international patent application by the Applicant No. WO 98/45335.

These sulphated derivatives have anticoagulative, non-thrombogenic, antiviral and anti-inflammatory properties, and it has been demonstrated that they inhibit platelet adhesion, aggregation and activation.

Moreover, the sulphated derivatives prove particularly advantageous in resisting the enzyme hyaluronidase, and they therefore ensure anticoagulant activity for far longer than heparin (G. Abatangelo et al., *Biomaterials* 18, 1997, 1411-1415).

However, not all the above derivatives as such cannot be processed in the form of biomaterials because the higher is the percentage of sulphation, the greater is their hydrophilia.

Therefore the need of novel bio- and haemocompatible compounds, which also have the advantageous properties of the sulphated hyaluronic acid and derivatives thereof, and can be used as such for the preparation of biomaterials and for the coating of biomedical objects, is deeply felt.

Summary of the invention

The present invention relates to polymers with a high degree of biocompatibility and haemocompatibility, constituted by a polyurethane bound covalently to a sulphated hyaluronic acid and derivatives thereof.

Said polymers maintain the mechanical characteristics (resistance to wear and tear, bending, elasticity, etc.) and the stability of polyurethane, also showing the anticoagulant activity, the effectiveness in inhibiting platelet adhesion, activation and aggregation, and the resistance to hyaluronidase of the sulphated hyaluronic acid and of the sulphated derivatives thereof.

Moreover, the derivatives according to the present invention, constituted by a polyurethane bound covalently to sulphated hyaluronic acid or its sulphated derivatives, show the considerable advantage of being easily mobilised on the polymer surface of biomedical objects, in most cases exploiting solubility in

organic solvents.

Indeed, the surface of an object made of polymeric material can be treated with the organic solution of the derivative triggering solubilization of the outer layers of the polymer and, due to the subsequent evaporation of the solvent, the derivative adheres to the surface, merging with the polymer material of which the object is made.

In view of the foregoing the present invention further relates to haemocompatible materials containing the polyurethane bound covalently to the sulphated hyaluronic acid or sulphated hyaluronic acid derivatives.

The present invention further relates to industrial or medical articles or devices coated with haemocompatible materials comprising the polyurethane bound covalently to the sulphated hyaluronic acid or sulphated hyaluronic derivatives.

Brief description of the drawings

Figure 1 shows the infra-red spectra of the O-sulphated hyaluronic acid with a degree of sulphation of 3.5, and of its polyurethane derivative in the dry and wet forms, as obtained in Example 1.

Figures 2, 3 and 4 show the SEM (Scanning Electron Microscope ; magnification = 1022x) images of the platelet adhesion test on the polyurethane derivative of O-sulphated hyaluronic acid obtained in Example 1.

Detailed description of the invention

By sulphated hyaluronic acid and sulphated hyaluronic acid derivatives we mean :

A_1) O-sulphated hyaluronic acid, and

A_2) O-sulphated hyaluronic acid derivatives,

both types being disclosed in WO 95/25751, we incorporate herewith by reference ;

B_1) N-sulphated hyaluronic acid, and

B_2) N-sulphated hyaluronic acid derivatives,

both types being disclosed in WO 98/45335, we incorporate herewith by reference.

In the O-sulphated derivatives of hyaluronic acid or hyaluronic acid derivatives of class A_1 and A_2 the number of O-sulphated groups is generally comprised

between 0.5 and 3.5.

In the N-sulphated hyaluronic acid B_1 or in the N-sulphated hyaluronic acid derivatives B_2 the glucosaminic portions of the repeating unit may be :

a) partially N-sulphated,

b) partially N-sulphated and partially O-sulphated, or

c) partially N-sulphated and totally O-sulphated,

wherein :

a) means a product obtained by means of a controlled sulphation reaction of the previously deacylated amino groups of glucosamine,

b) and c) mean a product obtained by a sulphation reaction in which, besides the previously mentioned deacylated amino groups of glucosamine, also the primary hydroxy functions of the same residue are involved, partially or totally respectively.

The hyaluronic acid derivatives used to prepare the sulphated compounds of classes A_2 and B_2 are selected among :

- the partial esters of hyaluronic acid containing at least one free carboxylic function and the remaining carboxylic functions being esterified with alcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic and heterocyclic series,
- the partial crosslinked esters containing at least one free carboxylic function and the remaining carboxylic functions being esterified with the alcoholic function of the same hyaluronic acid chain or of a different chain like those disclosed in USP No. 5,676,964, we incorporate herewith by reference,
- the partial crosslinked esters disclosed in USP No. 4,957,744 we incorporate herewith by reference containing at least one free carboxylic function and the remaining carboxylic functions reacted with a polyalcohol of the aliphatic, aromatic, arylaliphatic, heterocyclic series, and wherein a crosslinking is thereafter generated by means of spacer chains.

Any biocompatible polyurethane may be used for preparing the polyurethane bound covalently to sulphated hyaluronic acid. Preferred is the polyurethane present on the market with the trademark Pellethane®; particularly preferred is the polyurethane having an average molecular weight of 180000 Da, this polymer containing the repeating unit 4,4'-methylenebis (phenyl isocyanate).

The haemocompatible materials according to the present invention besides polyurethane bound covalently to sulphated hyaluronic acid may optionally further contain natural, synthetic or semisynthetic polymers and/or pharmaceutically active substances.

The pharmaceutically active substances that can be used are, for example, antibiotics, anti-infective, antimicrobial, antiviral, cytostatic, antitumoral, anti-inflammatory and wound healing agents, anaesthetics, cholinergic or adrenergic agonists and antagonists, antithrombotic, anticoagulant, haemostatic, fibrinolytic, thrombolytic agents, proteins and their fragments, peptides, polynucleotides, growth factors, enzymes and vaccines.

Among the natural polymers, it is possible to use, for example, collagen, coprecipitates of collagen and glycosamino glycans, cellulose, polysaccharides in the form of gels such as chitin, chitosan, pectin or pectic acid, agar, agarose, xanthane, gellan, alginic acid or alginates, polymannan or polyglycans, starch and natural gums.

The semisynthetic polymers, for example, can be chosen from the group consisting of collagen crosslinked with agents such as aldehydes or precursors of the same, dicarboxylic acid or the halides thereof, diamines, derivatives of cellulose, hyaluronic acid, chitin or chitosan, gellan, xanthane, pectin or pectic acid, polyglycans, polymannan, agar, agarose, natural gum and glycosamino glycans.

Lastly, among the synthetic polymers it is possible to use, for example, polylactic acid, polyglycolic acid or copolymers of the same or their derivatives, polydioxanes, polyphosphazenes, polysulphonic resins and PTFE.

The haemocompatible materials according to the present invention are preferably in the form of sponges, films, membranes, threads, tampons, non-woven fabrics, microspheres, nanospheres, gauzes, gels and guide channels.

The haemocompatible materials according to the present invention can be used in the cardiovascular field or in any application involving contact with the blood or with highly vascularised body tissues.

The above haemocompatible materials can be used to advantage in various

surgical fields, in internal, osteoarticular, neurological, anastomotic, viscoelastic, ophthalmic, oncological, aesthetic, plastic, otorhinolaryngological, abdominal-pelvic, urogynaecological and cardiovascular surgery, in the prevention of post-surgical adhesions and in the prevention of hypertrophic scarring.

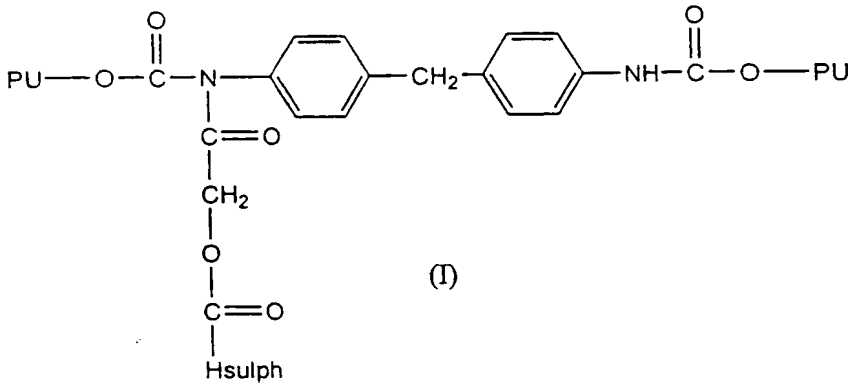
The haemocompatible materials according to the present invention can be used, besides in the surgical field, in haemodialysis, in cardiology, in dermatology, in ophthalmology, in otorhinolaryngology, in dentistry, in gynaecology, in urology and in extracorporeal blood circulation and oxygenation.

The above haemocompatible materials in their various forms can also be used to advantage as cell culture supports, such as for mesenchymal cells or mature cells to obtain connective, glandular and nerve tissue.

The haemocompatible materials can also be used in the processes of preparation and coating of articles or devices to be used both in the medical field and in industry, which show, due to this coating, biological characteristics on the surfaces.

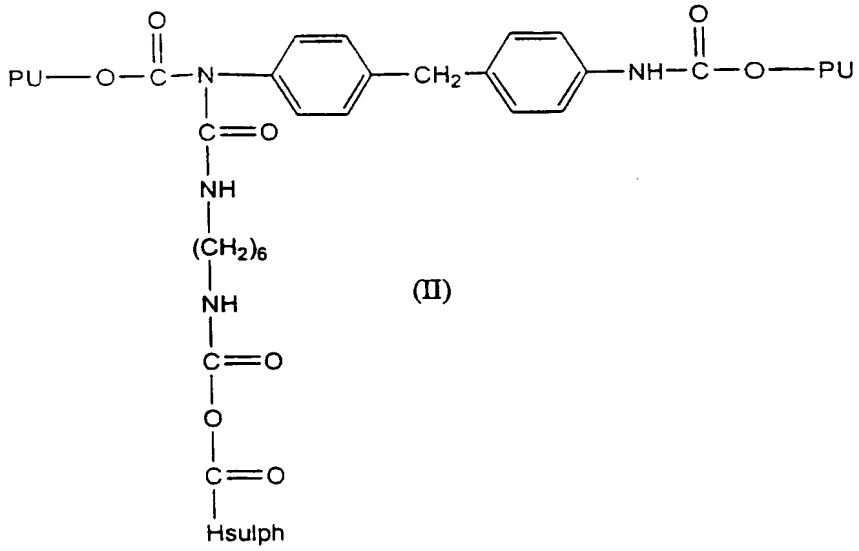
The objects that can be coated are, for example, catheters, guide channels, probes, cardiac valves, soft tissue prostheses, prostheses of animal origin such as cardiac valves from pigs, artificial tendons, bone and cardiovascular replacements, contact lenses, blood oxygenators, artificial kidneys, hearts, pancreases and livers, blood bags, syringes, surgical instruments, filtration systems, laboratory instruments, containers for cultures and for the regeneration of cells and tissues, supports for peptides, proteins and antibodies.

Particularly preferred polyurethane bound covalently to sulphated hyaluronic acid are those characterised by the following formula (I)



(I)

and formula (II)



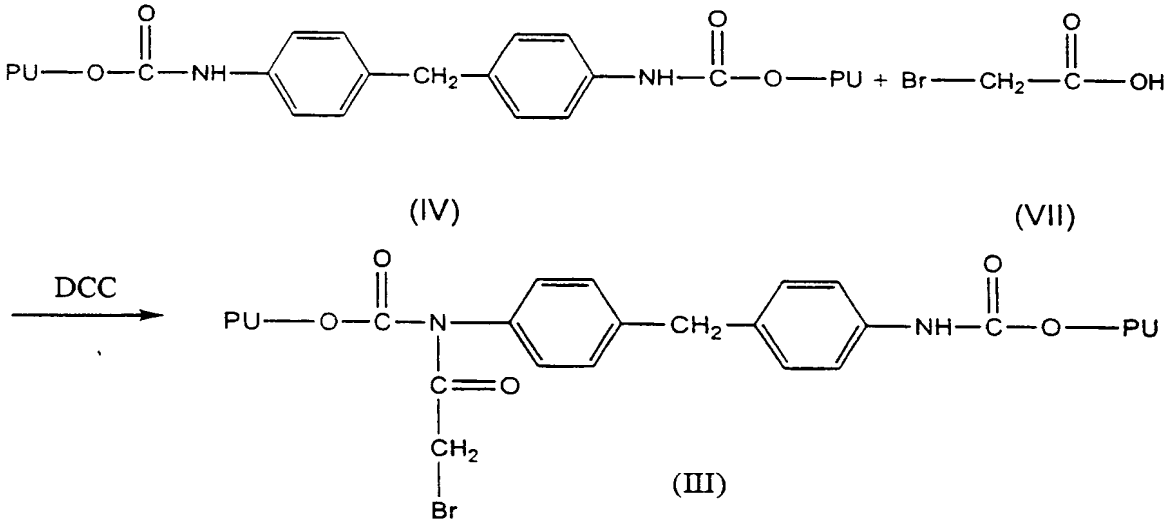
(II)

wherein PU is a residue of the polyurethane chain, and Hsulph is a residue of sulphated hyaluronic acid as in the above classes A_1 and B_1, or a sulphated hyaluronic acid derivative containing at least one free carboxylic function as in the above classes A_2 and B_2.

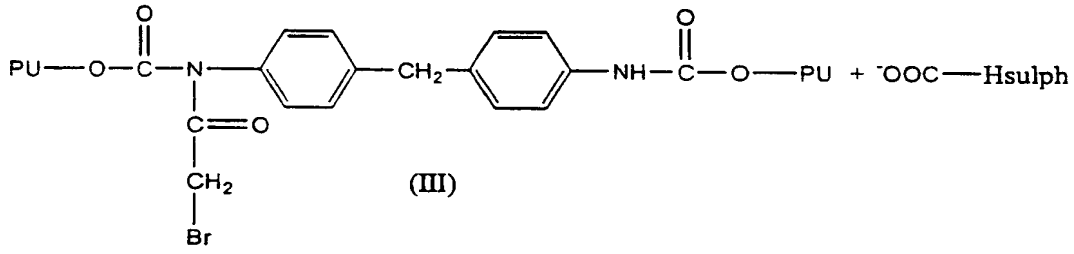
In particular, the process for preparing the polyurethane bound covalently to sulphated hyaluronic acid of formula (I) is obtained with a process comprising the following steps :

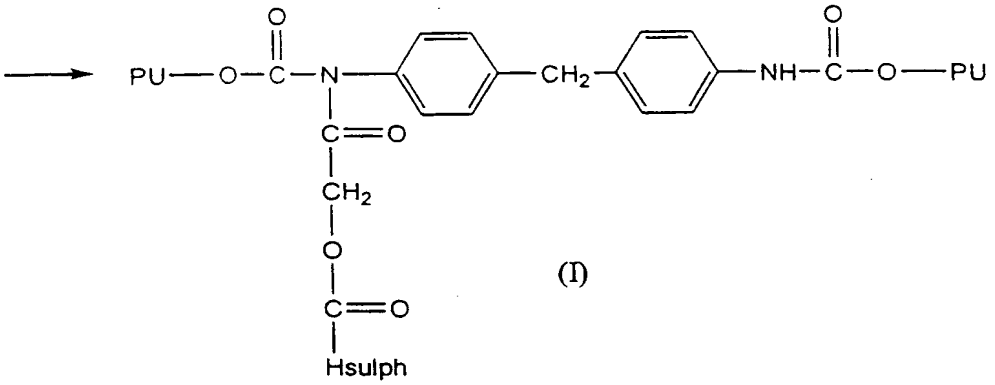
i) the polyurethane (IV) is reacted with bromoacetic acid (VII) in the presence of N,N'-dicyclohexylcarbodiimide (DCC), to obtain the adduct of formula (III)

according to the following reaction scheme :



ii) the adduct (III) coming from step i) is reacted with HOOC—Hsulph wherein Hsulph has the above meanings, thereby obtaining the compound of formula (I) according to the following scheme :





(I)

The reaction in step i) is typically carried out in an inert atmosphere and in an organic solvent, preferably in dimethylformamide (DMF).

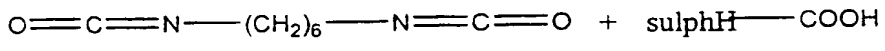
Before carrying out step ii) the reaction mixture coming from step i) is preferably filtered to separate the solution containing the desired product (III) from the precipitate of dicyclohexylurea which forms simultaneously.

Step ii) is preferably carried out in the presence of sodium bicarbonate.

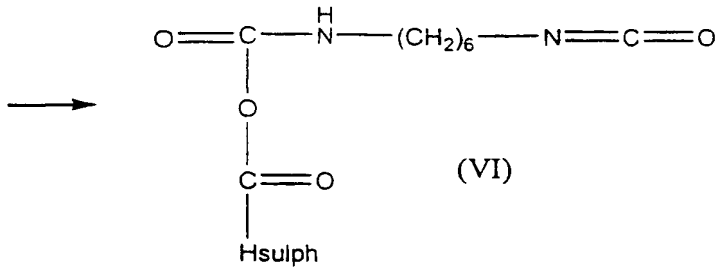
The reaction in step ii) is typically carried out in 24 hours at a temperature ranging from 25 to 45°C, and preferably at 25°C.

The polyurethane derivative of formula (II) can be obtained by a process comprising the following steps :

i') a sulphated hyaluronic acid or a sulphated hyaluronic acid derivative, wherein part or all of the carboxy groups of the glucuronic residue are in their acid form HOOC—Hsulph is reacted with hexamethylenediisocyanate (HMDI) (V), to obtain the adduct of formula (VI)

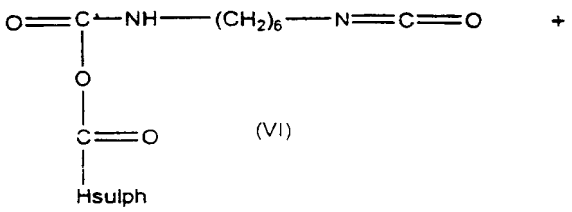


(V)

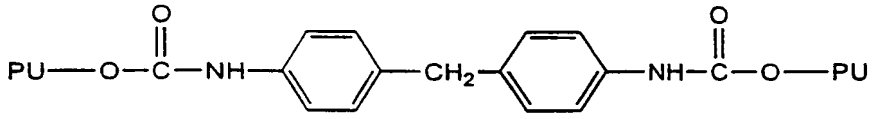


(VI)

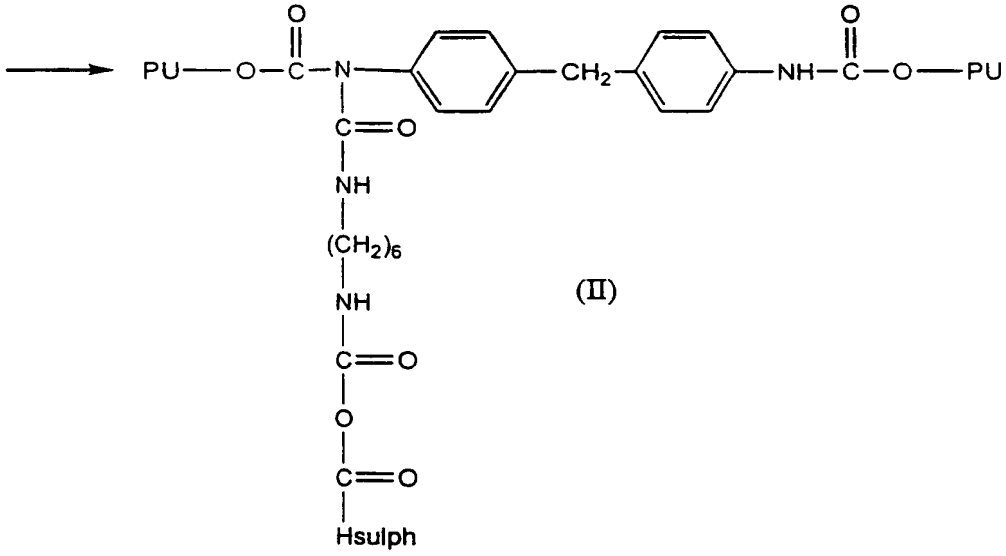
ii') the adduct (VI) coming from step i') is reacted with the polyurethane (IV) to obtain the desired product (II) according to the following scheme :



(VI)



(IV)



(II)

Both reactions in steps i') and ii') are typically carried out in an inert atmosphere,

by using DMF as the solvent.

The temperature in step ii') is kept in the range from 45 to 55°C for a time from 48 to 72 hours, while the mixture is left to react.

The following examples are given to provide non-limiting illustrations of the present invention.

EXAMPLE 1

Polyurethane covalently bound to O-sulphated hyaluronic acid of formula (I) (PUBRAC-1)

30 ml of a 10% (w/v) solution in DMF of Pellethane® are supplemented with 1.5 g of DCC while stirring.

Once the DCC has dissolved, 1.8 g of bromoacetic acid dissolved in a minimal quantity of DMF are added drop by drop.

After approximately 30 minutes the solution is filtered to separate it from the white dicyclohexylurea precipitate.

1 g of O-sulphated hyaluronic acid sodium salt (molecular weight 200 kDa and degree of sulphation 3.5) is dissolved in 60 ml of water, and this solution has percolated along the length of a ion exchange column, packed with 75 ml of a sulphonic resin in the form of tetrabutilammonium salt.

This resin has been prepared by the means of activation of a protonated sulphonic resin with a tetrabutilammonium hydroxide 40% w/v solution.

The solution containing the O-sulphated hyaluronic acid tetrabutilammonium salt coming from the column has been collected, then freeze dried.

200 mg of the so-obtained O-sulphated hyaluronic acid tetrabutilammonium salt and 2 g of sodium bicarbonate are added to the above polyurethane solution in DMF.

The mixture is left to react for 24 hours while being stirred at a temperature of 25°C.

If any precipitate has formed the reaction mixture is filtered again, then cast in Petri dishes.

We report hereafter in Figure 1 the infra-red spectra of the sulphated hyaluronic acid with a degree of sulphation of 3.5, and of its polyurethane derivative in the

dry and wet forms, obtained as above illustrated.

The polyurethane derivative in its dry state presents the typical spectrum of polyurethane not modified with sulphated hyaluronic acid, whereas in its wet state, peaks of between 3600 and 2800 cm^{-1} and at 1654 cm^{-1} can be seen as relative to the functional groups of the sulphated hyaluronic acid.

EXAMPLE 2

Polyurethane covalently bound to N-sulphated hyaluronic acid of formula (I) (PUBRAC-2)

30 ml of a 10% (w/v) solution in DMF of Pellethane® are supplemented with 1.5 g of DCC under stirring.

Once the DCC has dissolved, 1.8 g of bromoacetic acid dissolved in a minimal quantity of DMF is added drop by drop.

30 to 40 minutes later, the solution is filtered to separate it from the white precipitate of dicyclohexylurea.

This solution is supplemented with 2 g of sodium bicarbonate and 200 mg of N-sulphated hyaluronic acid tetrabutilammonium salt obtained starting from N-sulphated hyaluronic acid sodium salt (molecular weight 200 KDa and 30% sulphation) as described in Example 1 for the corresponding O-sulphated compound.

The reaction mixture is then left to react for 24 hours under stirring at a temperature of 25°C.

It is filtered again, and then cast in Petri dishes.

EXAMPLE 3

Polyurethane covalently bound to O-sulphated hyaluronic acid of formula (I) (PUBRAC-3)

2 g of DCC are added in an inert atmosphere to 25 ml of a 10% (w/v) solution in DMF of Pellethane® while stirring.

Once the DCC has dissolved, 1.8 g of bromoacetic acid dissolved in a minimal quantity of DMF are added drop by drop.

After approximately 30 minutes the solution is filtered to separate it from the white precipitate of dicyclohexylurea.

To the so-obtained solution 250 mg of O-sulphated hyaluronic acid tetrabutilammonium salt, prepared starting from the corresponding sodium salt (molecular weight 200 KDa and degree of sulphation 3.5) as described above in Example 1, and 2 g of sodium bicarbonate are added, then the mixture is left to react for 24 hours while being stirred at a temperature of 45°C.

If any precipitate has formed the reaction mixture is filtered again, then cast in Petri dishes.

EXAMPLE 4

Purification of polyurethane covalently bound to sulphated hyaluronic acid of formula (I) obtained according to Example 3 (PUBRAC Ris THF)

The preparation procedure as described in Example 3 is carried out once again, but the reaction product is dissolved in THF before cast in Petri dishes.

EXAMPLE 5

Purification of polyurethane covalently bound to sulphated hyaluronic acid of formula (I) obtained according to Examples 1-4 (PUBRAC)

Before cast in Petri dishes, the reaction product as obtained in Examples 1-4 is first washed with acetone, then 2-3 washing with a 10% solution of NaCl are performed.

EXAMPLE 6

Polyurethane covalently bound to O-sulphated hyaluronic acid of formula (II) (PUHMDI-6)

O-sulphated hyaluronic acid is obtained starting from the corresponding sodium salt (molecular weight 200 kDa and degree of sulphation 3.5) as described above in Example 1, and a complete protonation of its carboxy group is performed bringing the tetrabutilammonium salt solution coming from the column to pH = 3-4, before freeze drying.

300 mg of the so-obtained O-sulphated hyaluronic acid are dissolved in the minimal quantity of DMF (approximately 10 ml).

Once solubilization is complete, the solution is placed in a flask containing 200 µl of HMDI under stirring and in an inert atmosphere.

30 minutes later, 10 ml of a 10% (w/v) Pellethane® solution in DMF are added.

The solution is left under stirring and in an inert atmosphere at a temperature of 45-50°C for 3 days. It is then cast in Petri dishes.

EXAMPLE 7

Polyurethane covalently bound to O-sulphated hyaluronic acid of formula (II) (PUHMDI-7)

O-sulphated hyaluronic acid is obtained starting from the corresponding sodium salt (molecular weight 200 kDa and degree of sulphation 3.5) as described above in Example 1, and a complete protonation of its carboxy group is performed bringing the tetrabutilammonium salt solution coming from the column to pH = 3-4, before freeze drying.

250 mg of the so-obtained O-sulphated hyaluronic acid are dissolved in the minimal quantity of DMF (approximately 10 ml), then the solution is poured under stirring and in an inert atmosphere into a flask containing 200 µl of HMDI.

30 minutes later, 25 ml of a 10% (w/v) solution in DMF of Pellethane® are added to the reaction mixture preserving an inert atmosphere.

The solution is left under stirring and in an inert atmosphere at a temperature of 55°C for 48 hours. It is then cast in Petri dishes.

EXAMPLE 8

Purification of polyurethane covalently bound to O-sulphated hyaluronic acid of formula (II) obtained according to Examples 6 and 7 (PUHMDI)

Before cast in Petri dishes, the reaction product as obtained in Examples 6 and 7 is washed with a 10% solution of NaCl for 2-3 times.

EXAMPLE 9

Test of platelet adhesion on the material obtained according to Example 1 (PUBRAC-1).

Blood was drawn from a healthy, non-smoking donor who had taken no drugs for a fortnight before. Platelet-rich plasma (PRP) was obtained by centrifuging the whole blood at 250 rpm for 25 minutes at room temperature.

1 ml of PRP was placed in contact with each sample (0.5 cm x 0.5 cm) of the test polymer and these were then left for 3 hours at room temperature in order to favour platelet adhesion. The samples were then washed in PBS (phosphate

buffer solution) to remove any platelets which had not adhered to the surface, and then incubated in a solution of glutaraldehyde at 2.5% (v/v) in 100 mM sodium cacodylate for 30 seconds.

Subsequently, the films were washed in cacodylate of sodium, 100 mM, for 30 seconds, rinsed in distilled water and left in the first dehydrating solution (70% v/v of ethanol in distilled water) for 15 minutes. The samples were then transferred to the second dehydrating solution (90% v/v of ethanol in distilled water) for 15 minutes and lastly in absolute ethanol for another 15 minutes.

All the samples were then dehydrated in a vacuum for 12 hours, metallized with gold and analysed with a scanning electron microscope (SEM) (Figures 2, 3 and 4).

As can be seen from figures 2, 3 and 4, the surface of the material is morphologically irregular and characterised by the presence of numerous slits of varying sizes. Despite these irregularities, 90% of the material presents no phenomena of platelet adhesion.

Only on the remaining 10% of the surface can the presence of platelets be observed, which in some cases form small clusters while in others they appear to maintain their individual character even though they have lost the discoid shape typical of non-activated platelets, and they have extruded pseudopods with which they cling to the surface.

EXAMPLE 10

Test of whole blood coagulation on the material obtained according to Examples 3 (PUBRAC-3) and 4 (PUBRAC Ris THF).

This test was performed on PUBRAC-3 and PUBRAC Ris THF using blood from a single donor.

5 ml of blood was placed in contact with a sample (0.5 cm x 0.5 cm) of the following materials :

control	polystyrene
PU	Pellethane®
PUBRAC-3	polyurethane derivative according to Example 3
PUBRAC Ris THF	polyurethane derivative according to Example 4

The samples were left at room temperature and the time necessary to achieve blood coagulation is then measured.

The results are reported in the following table :

Table 1

SAMPLE	COAGULATION TIME (minutes)
Control (polystyrene)	25 ± 2
PU	26 ± 2
PUBRAC-3	> 120
PUBRAC Ris THF	> 120

Table 1 shows that the polyurethane derivatives according to the present invention have an anticoagulant activity at least equal to that of polyurethane, and even much higher than that for the polyurethane derivative obtained according to Example 3, which shows a coagulation time getting over 2 hours.

EXAMPLE 11

Thrombin time measured by using the material obtained according to Example 3 (PUBRAC-3) and 7 (PUHMDI-7).

The ability of the derivatives according to the present invention in increasing blood coagulation time is measured by the thrombin time test conducted with a coagulometer.

An assessment is made of the time it takes to transform fibrinogen into fibrin after the addition of an excess of thrombin in a blood sample in the presence of the polymer. A result of over 120 seconds is no longer significant.

The results are reported in the following table:

Table 2

SAMPLES	THROMBIN TIME (seconds)
Control (polystyrene)	12.1 ± 0.9
PU	12.5 ± 0.4
PUBRAC-3 air side (∅ 0.8 cm)	> 120
PUBRAC-3 glass side (∅ 0.8 cm)	> 120
PUBRAC-3 glass side (0.8 cm x 0.5 cm)#	26.2 ± 3.8
PUBRAC-3 air side (0.8 cm x 0.5 cm)#	15.2 ± 0.2
PUHMDI-7(0.8 cm x 0.5 cm)	16.3 ± 0.2

thrombin time determined on plasma after 10 minutes contact with the polyurethane derivative at 37°C

The table shows that the anticoagulant activity occurs on the side of the film which is in contact with the glass because the polar environment causes the sulphated hyaluronic acid group to be exposed on the surface, while different results are observed on the side which is in contact with the air.

EXAMPLE 12

Reptilase time measured by using the material obtained according to Examples 3 (PUBRAC-3) and 4 (PUBRAC Ris THF).

Reptilase, a fraction extracted from the venom of the South American snake Bothrox atrox, is an enzyme that clots fibrinogen by splitting off its fibrinopeptide A.

The reptilase time is determined by incubating 0.3 ml of human plasma on the round samples (diameter 0.8 cm) of PUBRAC-3 and PUBRAC Ris THF at 37 °C for 2 minutes, then adding Reptilase Reactive (function of thrombin extracts from Bothrox Atrox venom, Haemodiagnostica Diagnostica Stago, Boehringer Mannheim), and measuring the clotting time automatically (Elvi Digiclot 2 Coagulometer, Logos S.p.A., Milan, Italy). Table 3 shows the effects of the materials obtained according to Examples 3 and 4 on reptilase time.

Table 3

SAMPLE	REPTILASE TIME
Control (polystyrene)	16.20 ± 0.05
PUBRAC-3	15.2 ± 0.2
PUBRAC Ris THF	16.65 ± 0.05

The data in Table 3 show that the materials obtained according to Examples 3 and 4 have moderate and not very significant effects on reptilase time.

EXAMPLE 13

Thrombin inhibition measured by using the material obtained according to Example 3 (PUBRAC-3)

The thrombin inhibition in plasma and in the presence of purified molecules, i.e. antithrombin III (AT III) and heparin cofactor (HC II), were studied for the material as obtained in Example 3 (PUBRAC-3), in order to investigate the manner in which the derivatives of the present invention exert their anticoagulant activity.

Selected donors were normal, healthy subjects who had fasted for more than 8 hours and had not taken any medication for at least 14 days.

Blood samples were drawn in 3.8% (w/v) tri-sodium citrate as anticoagulant at a ratio of 9 parts blood to 1 part citrate. The samples were centrifuged at 3500 rpm for 15 minutes to obtain platelet poor plasma (PPP). Pooled citrated plasma was prepared from 10-12 normal drug free volunteers and stored in aliquots at -80°C.

AT III (1 U.I./ml) and HC II (Heparin Cofactor II purchased by Calbiochem, USA) were reconstituted from lyophilised powder with sterile water and used immediately. 32.4 mg of human fibrinogen (molecular weight ≈ 341,000, Calbiochem, USA) was dissolved in 6 ml of a physiological solution (0.9% NaCl, pH = 7.4), then 0.2 ml of this solution were placed in contact with a sample of PUBRAC-3 (∅ 0.7 cm).

0.2 ml of AT III or 0.2 ml of HC II or 0.2 ml of PBS was then added to the above sample.

The thrombin time with or without AT III and HC II was determined manually by adding 0.2 ml of thrombin (Human Thrombin purchased by Böheringer Mannheim, Germany) to 0.2 ml of the above samples.

The results are summarised in the following table :

Table 4

SAMPLE	Thrombin Time (sec.) without AT III and HC II	Thrombin Time (sec.) with AT III	Thrombin Time (sec.) with HC II
PU	8.4 ± 0.4	8.1 ± 0.2	18.5 ± 1.3
PUBRAC-3	67.3 ± 3.3	63.3 ± 3.3	> 120

The above experiment was performed both with and without AT III and the results obtained were approximately the same in both cases, thus demonstrating that presumably the inactivation of thrombin by the polyurethane derivatives of the present invention is not mediated by AT III.

Moreover, the above results show the ability of the present derivatives to accelerate the thrombin inhibition mediated by HC II.

In conclusion, the thrombin was inhibited by the present polyurethane derivatives both via HC II and via direct interaction.

CLAIMS

1. A polyurethane bound covalently to sulphated hyaluronic acid or to a sulphated hyaluronic acid derivative.

2. The polyurethane according to claim 1, wherein the starting polyurethane comprises the repeating unit 4,4'-methylenebis (phenyl isocyanate).

3. The polyurethane according to any of claims 1 and 2, wherein the starting sulphated hyaluronic acid is selected from the group consisting of :

A_1) O-sulphated hyaluronic acid, and

B_1) N-sulphated hyaluronic acid.

4. The polyurethane according to any of claims 1 and 2, wherein the starting sulphated hyaluronic acid derivative is selected from the group consisting of :

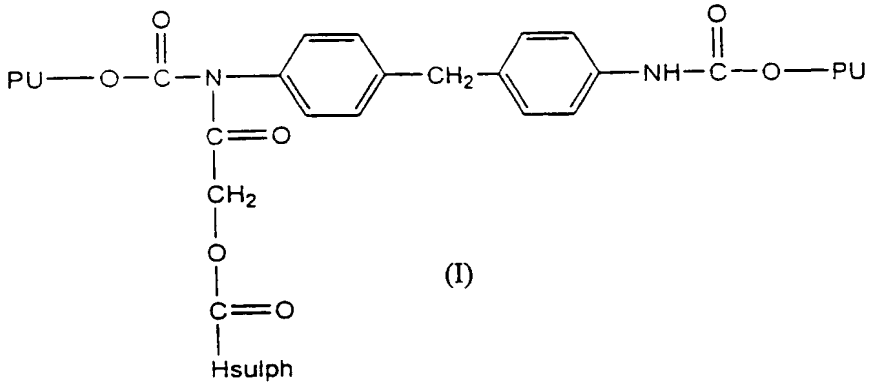
A_2) O-sulphated hyaluronic acid derivative, and

B_2) N-sulphated hyaluronic acid derivative.

5. The polyurethane according to claim 4, wherein the hyaluronic acid derivatives used to prepare the starting sulphated hyaluronic acid A_2 and B_2 are selected from the group consisting of :

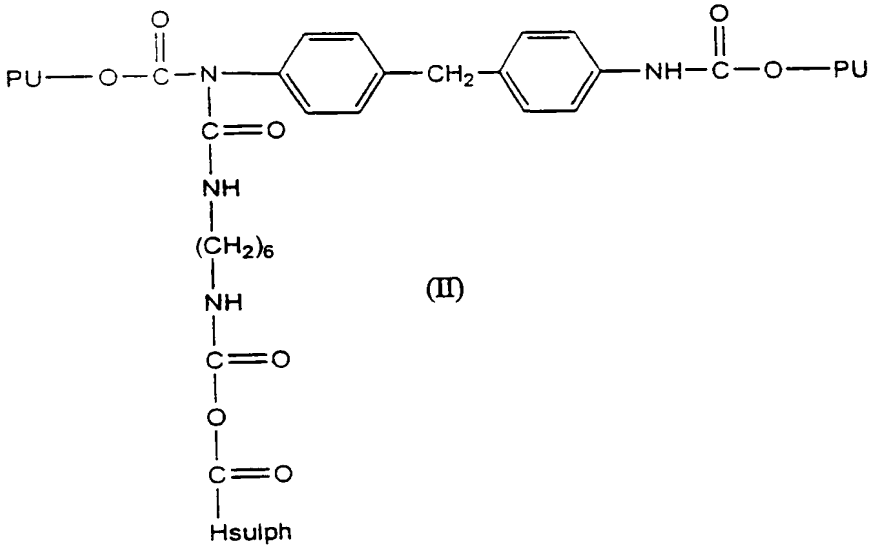
- the partial esters of hyaluronic acid containing at least one free carboxylic function and the remaining carboxylic function esterified with alcohols of the alifatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series,
- the partial crosslinked esters containing at least one free carboxylic function and the remaining carboxylic functions are esterified with the alcoholic function of the same hyaluronic acid or of a different chain,
- the partial crosslinked esters containing at least one free carboxylic function reacted with a polyalcohol of the aliphatic, aromatic, arylaliphatic, heterocyclic series, and wherein crosslinking is thereafter generated by means of spacer chains.

6. The polyurethane according to any of claims 1-5 of formula (I)



(I)

or formula (II)



(II)

wherein PU is a residue of the polyurethane chain, Hsulph is a residue of the sulphated hyaluronic acid or a sulphated hyaluronic acid derivative containing at least one free carboxylic function.

7. A process for preparing the polyurethane of formula (I) comprising the following steps :

i) the polyurethane (IV) is reacted with bromoacetic acid (VII) in the presence of N,N'-dicyclohexylcarbodiimide (DCC), to obtain the adduct of formula (III) ;

ii) the adduct (III) coming from step i) is reacted with HOOC—Hsulph, thereby

chitosan, gellan, xanthane, pectin or pectic acid, polyglycans, polymannan, agar, agarose, natural gum and glycosamino glycans.

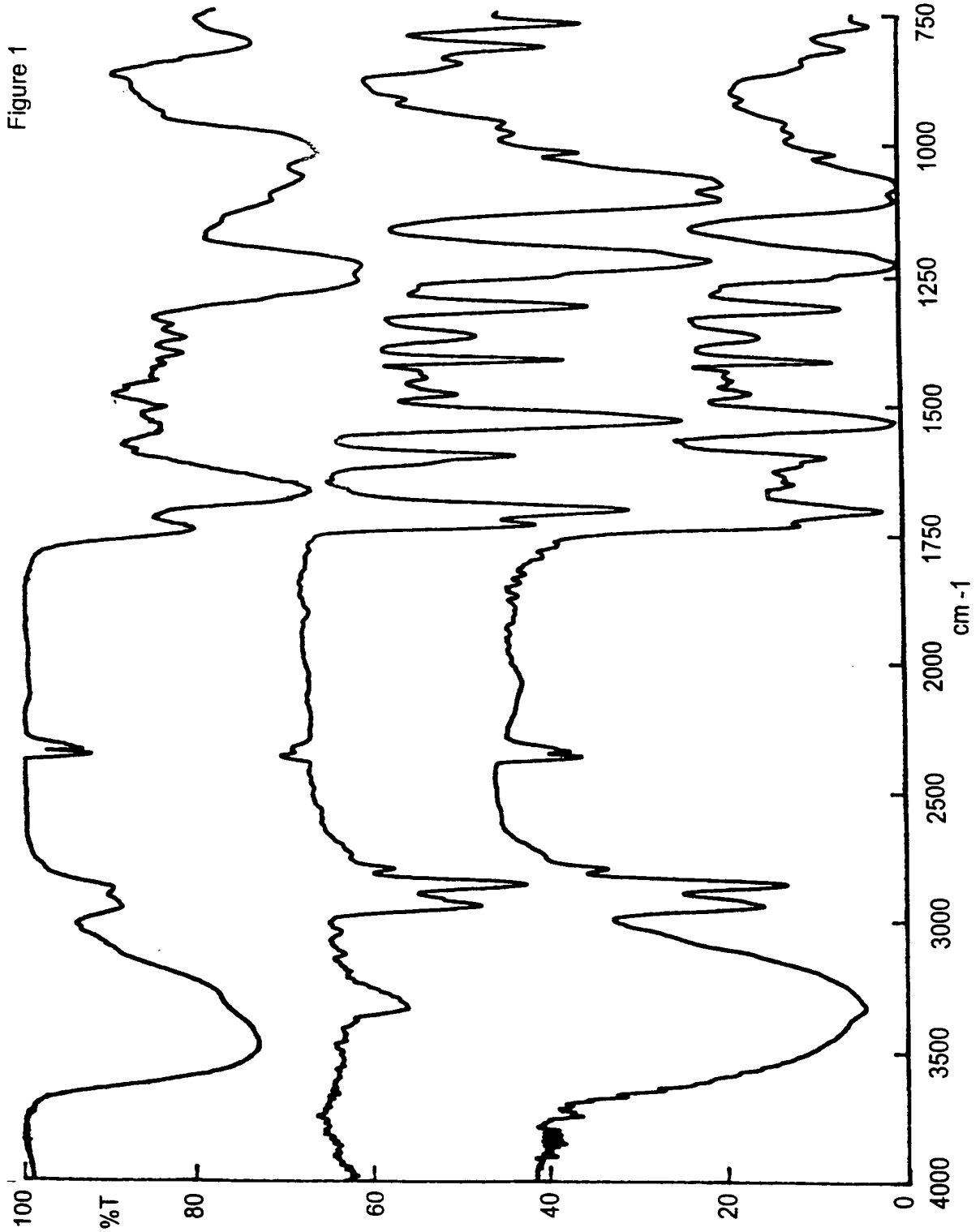
16. The haemocompatible material according to claim 13, wherein said synthetic polymer is selected from the group consisting of polylactic acid, polyglycolic acid or copolymers of the same or their derivatives, polydioxanes, polyphosphazenes, polysulphonic resins and PTFE.

17. The haemocompatible material according to any of claims 9-16, in the form of sponges, films, membranes, threads, tampons, non-woven fabrics, microspheres, nanospheres, gauzes, gels and guide channels.

18. Industrial or medical articles or devices made with or coated with the haemocompatible material according to any of claims 9-16.

19. The industrial or medical articles or devices according to claim 18, wherein said devices are selected from the group consisting of catheters, guide channels, probes, cardiac valves, soft tissue prostheses, prostheses of animal origin such as cardiac valves from pigs, artificial tendons, bone replacements or cardiovascular prostheses, contact lenses, blood oxygenators, artificial kidneys, hearts, pancreas and livers, blood bags, syringes, surgical instruments, filtration systems, laboratory instruments, containers for cultures and for cell and tissue regeneration, supports for peptides, proteins and antibodies.

Figure 1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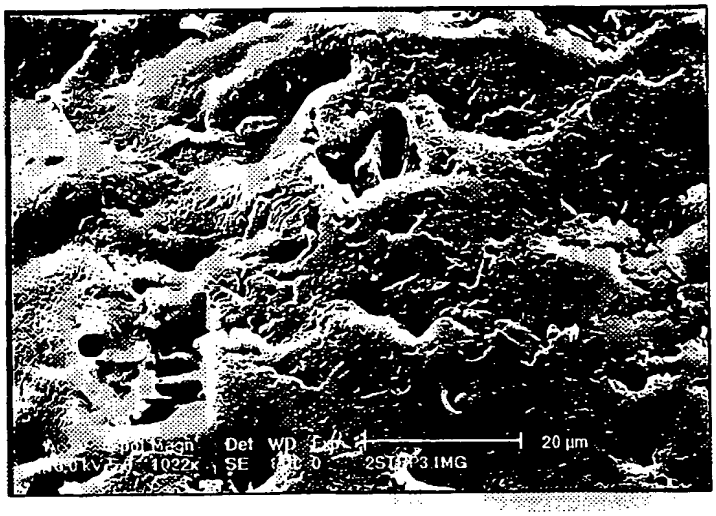
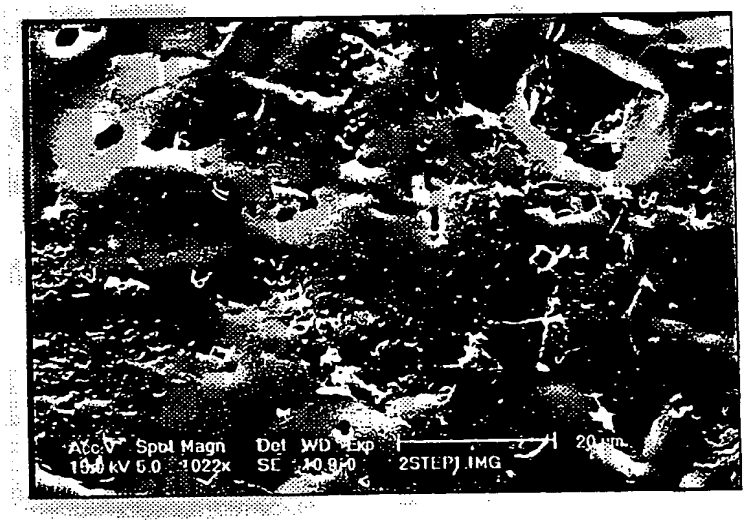


Figure 2

Figure 3



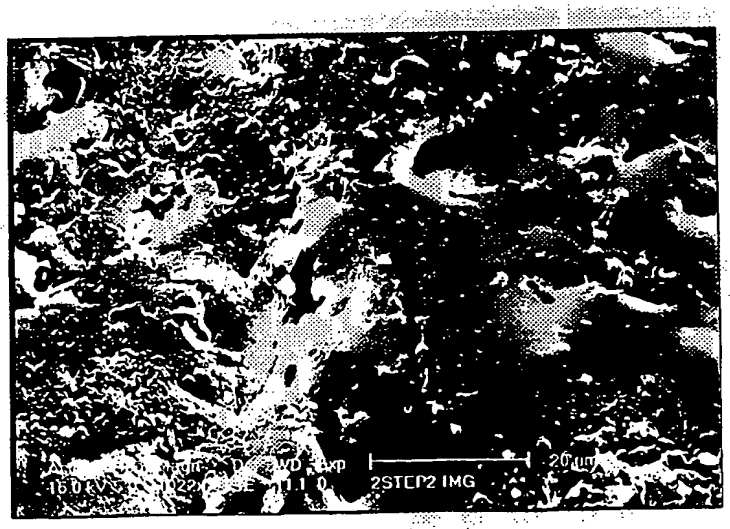


Figure 4

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]/EP 99/01191

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C08G18/83 A61L27/00 A61L33/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C08G A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4 487 865 A (BALAZS ENDRE A ET AL) 11 December 1984 see examples 1,2	1-19
Y	WO 95 25751 A (FIDIA ADVANCED BIOPOLYMERS SRL ;BARBUCCI STEFANIA & HF (IT); BARBU) 28 September 1995 cited in the application see claims 1,5	1-19
A	US 4 944 767 A (BARBUCCI ROLANDO ET AL) 31 July 1990 cited in the application see claims 1-22	1

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 July 1999	09/07/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hoffmann, K

2

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MARCONI W: "NEW POLYURETHANE COMPOSITIONS CONTAINING HIGH AMOUNTS OF COVALENTLY BONDED HEPARIN" MAKROMOLEKULARE CHEMIE, MACROMOLECULAR CHEMISTRY AND PHYSICS, vol. 194, no. 5, 1 May 1993, pages 1347-1356, XP000367487 cited in the application see page 1347 - page 1348	1-19

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
[redacted]/EP 99/01191

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4487865 A	11-12-1984	AU 551704 B	08-05-1986
		AU 3209284 A	20-06-1985
		CA 1223383 A	23-06-1987
		DE 3434123 A	27-06-1985
		FR 2556732 A	21-06-1985
		GB 2151247 A,B	17-07-1985
		JP 1481361 C	10-02-1989
		JP 60130638 A	12-07-1985
		JP 63023223 B	16-05-1988
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		AU 2072795 A	09-10-1995
		CA 2163337 A	28-09-1995
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		JP 9510493 T	21-10-1997
US 4944767 A	31-07-1990	DK 25387 A	17-07-1987
		EP 0230281 A	29-07-1987
		JP 62215621 A	22-09-1987

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

INFORMATION CONCERNING ELECTED OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

To:

GERVASI, Gemma
Notarbartolo & Gervasi S.p.A.
Corso di Porta Vittoria, 9
I-20122 Milan
ITALIE

Date of mailing (day/month/year) 29 October 1999 (29.10.99)	
Applicant's or agent's file reference 1845PTWO	**IMPORTANT INFORMATION**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/EP99/01191	24 February 1999 (24.02.99)	25 February 1998 (25.02.98)

Applicant
FIDIA ADVANCED BIOPOLYMERS S.r.l. et al

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

   AP :GH,GM,KE,LS,MW,SD,SZ,UG,ZW
   EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
   National :AU,BG,BR,CA,CN,CZ,DE,IL,JP,KP,KR,MN,NO,NZ,PL,RO,RU,SE,SK,US

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

   EA :AM,AZ,BY,KG,KZ,MD,RU,TJ,TM
   OA :BF,BJ,CF,CG,CI,CM,GA,GN,GW,ML,MR,NE,SN,TD,TG
   National :AL,AM,AT,AZ,BA,BB,BY,CH,CU,DK,EE,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,
   IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MW,MX,PT,SD,SG,SI,SL,TJ,TM,
   TR,TT,UA,UG,UZ,VN,YU,ZW

3. The applicant is reminded that he must enter the "national phase" **before the expiration of 30 months from the priority date** before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing , if prescribed, a translation of the international application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 36(3)(b) and Rule 74.1).

   Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

   The entry into the European regional phase is postponed **until 31 months from the priority date** for all States designated for the purposes of obtaining a European patent.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:
A. Karkachi

Telephone No. (41-22) 338.83.38

WO 99/43728
PCT/EP99/01191

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
GERVASI, Gemma
Notarbartolo & Gervasi S.p.A.
Corso di Porta Vittoria, 9
I-20122 Milan
ITALIE

Date of mailing (day/month/year) 02 September 1999 (02.09.99)		IMPORTANT NOTICE
Applicant's or agent's file reference 1845PTWO		
International application No. PCT/EP99/01191	**International filing date** (day/month/year) 24 February 1999 (24.02.99)	**Priority date** (day/month/year) 25 February 1998 (25.02.98)
Applicant FIDIA ADVANCED BIOPOLYMERS S.r.l. et al		

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,CN,EP,IL,JP,KP,KR,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CU,CZ,DE,DK,EA,EE,ES,FI,GB,GD,GE,GH,GM,HR,HU,
   ID,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NO,NZ,OA,PL,PT,RO,RU,SD,
   SE,SG,SI,SK,SL,TJ,TM,TR,TT,UA,UG,UZ,VN,YU,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   02 September 1999 (02.09.99) under No. WO 99/43728

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

Continuation of Form PCT/IB/308

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

Date of mailing (day/month/year) 02 September 1999 (02.09.99)	**IMPORTANT NOTICE**
Applicant's or agent's file reference 1845PTWO	**International application No.** PCT/EP99/01191

The applicant is hereby notified that, at the time of establishment of this Notice, the time limit under Rule 46.1 for making amendments under Article 19 has not yet expired and the International Bureau had received neither such amendments nor a declaration that the applicant does not wish to make amendments.

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To:
NOTARBARTOLO & GERVASI S.P.A.
Corso di Porta Vittoria, 9
I-20122 Milano
ITALIE

PCT

NOTIFICATION OF THE INTERNATIONAL APPLICATION NUMBER AND OF THE INTERNATIONAL FILING DATE

(PCT Rule 20.5(c))

Date of mailing *(day/month/year)*	15.04.99
Applicant's or agent's file reference: 1845PTWO	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP 99/01191	24/02/1999	25/02/1998

Applicant: FIDIA ADVANCED BIOPOLYMERS SRL

Title of the invention:

1. The applicant is hereby notified that the international application has been accorded the international application number and the international filing date indicated above.
2. The applicant is further notified that the record copy of the international application was transmitted to the International Bureau on the above date of mailing.
3. ☐ Other:

* The International Bureau monitors the transmittal of the record copy by the receiving Office and will notify the applicant (with Form PCT/IB/301) of its receipt. Should the record copy not have been received by the expiration of 14 months from the priority date, the International Bureau will notify the applicant (Rule 22.1(c)).

Name and mailing address of the receiving Office	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mme. M. Luyten 15. 04. 99

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No. PCT/EP 99 / 01191

International Filing Date 24 FEB 1999 (24.02.1999)

Name of receiving Office and "PCT International Application": EUROPEAN PATENT OFFICE PCT INTERNATIONAL APPLICATION

Applicant's or agent's file reference *(if desired) (12 characters maximum)*: 1845PTWO

Box No. I TITLE OF INVENTION SULPHATED HYALURONIC ACID AND SULPHATED DERIVATIVES THEREOF COVALENTLY BOUND TO SYNTHETIC POLYMERS, AND THE PROCESS FOR THEIR PREPARATION

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

FIDIA ADVANCED BIOPOLYMERS S.r.l.
Via De' Carpentieri 3
72100 BRINDISI - ITALY

[ ] This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: IT

State *(that is, country)* of residence: IT

This person is applicant for the purposes of: [ ] all designated States [X] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

BARBUCCI Rolando
Piazza 3 Luglio 6B
53100 SIENA - ITALY

This person is:
[ ] applicant only
[X] applicant and inventor
[ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: IT

State *(that is, country)* of residence: IT

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [X] the United States of America only [ ] the States indicated in the Supplemental Box

[X] Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: [X] agent [ ] common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

GERVASI Gemma
NOTARBARTOLO & GERVASI S.p.A.
Corso di Porta Vittoria 9
20122 MILAN - ITALY

Telephone No. 02/541799.1

Facsimile No. 02/54179920

Teleprinter No.

[ ] **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

CONSUMI Marco
S. Eugenia 83
53100 SIENA - IT ~~ALY~~

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: IT

State *(that is, country)* of residence: IT

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [X] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

MAGNANI Agnese
Località Agresto 391
53010 S. ROCCO A PILLI (Province of SIENA) - IT ~~ALY~~

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: IT

State *(that is, country)* of residence: IT

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [X] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

CALLEGARO Lanfranco
Via Monte Grappa 6
36016 THIENE (Province of VICENZA) - IT ~~ALY~~

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: IT

State *(that is, country)* of residence: IT

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [X] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

[ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- [X] **AP** **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- [X] **EA** **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- [X] **EP** **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- [X] **OA** **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- [X] **AL** Albania
- [X] **AM** Armenia
- [X] **AT** Austria
- [X] **AU** Australia
- [X] **AZ** Azerbaijan
- [X] **BA** Bosnia and Herzegovina
- [X] **BB** Barbados
- [X] **BG** Bulgaria
- [X] **BR** Brazil
- [X] **BY** Belarus
- [X] **CA** Canada
- [X] **CH and LI** Switzerland and Liechtenstein
- [X] **CN** China
- [X] **CU** Cuba
- [X] **CZ** Czech Republic
- [X] **DE** Germany
- [X] **DK** Denmark
- [X] **EE** Estonia
- [X] **ES** Spain
- [X] **FI** Finland
- [X] **GB** United Kingdom
- [X] **GD** Grenada
- [X] **GE** Georgia
- [X] **GH** Ghana
- [X] **GM** Gambia
- [X] **HR** Croatia
- [X] **HU** Hungary
- [X] **ID** Indonesia
- [X] **IL** Israel
- [X] **IN** India
- [X] **IS** Iceland
- [X] **JP** Japan
- [X] **KE** Kenya
- [X] **KG** Kyrgyzstan
- [X] **KP** Democratic People's Republic of Korea
- [X] **KR** Republic of Korea
- [X] **KZ** Kazakhstan
- [X] **LC** Saint Lucia
- [X] **LK** Sri Lanka
- [X] **LR** Liberia
- [X] **LS** Lesotho
- [X] **LT** Lithuania
- [X] **LU** Luxembourg
- [X] **LV** Latvia
- [X] **MD** Republic of Moldova
- [X] **MG** Madagascar
- [X] **MK** The former Yugoslav Republic of Macedonia
- [X] **MN** Mongolia
- [X] **MW** Malawi
- [X] **MX** Mexico
- [X] **NO** Norway
- [X] **NZ** New Zealand
- [X] **PL** Poland
- [X] **PT** Portugal
- [X] **RO** Romania
- [X] **RU** Russian Federation
- [X] **SD** Sudan
- [X] **SE** Sweden
- [X] **SG** Singapore
- [X] **SI** Slovenia
- [X] **SK** Slovakia
- [X] **SL** Sierra Leone
- [X] **TJ** Tajikistan
- [X] **TM** Turkmenistan
- [X] **TR** Turkey
- [X] **TT** Trinidad and Tobago
- [X] **UA** Ukraine
- [X] **UG** Uganda
- [X] **US** United States of America
- [X] **UZ** Uzbekistan
- [X] **VN** Viet Nam
- [X] **YU** Yugoslavia
- [X] **ZW** Zimbabwe

Check-boxes reserved for designating States (for the purposes f a national patent) which have become party to the PCT after issuance of this sheet:

- [ ] .....
- [ ] .....
- [ ] .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all ther designations which w uld be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmati n and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

	Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1)	25 February 1998 (25.02.98)	PD98A000037	ITALY [IT]		
item (2)					
item (3)					

☐ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): _____

** Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*
ISA /

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*
Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	:	4
description (excluding sequence listing part)	:	19
claims	:	4
abstract	:	1
drawings	:	2
sequence listing part of description	:	
Total number of sheets	:	30

This international application is **accompanied by** the item(s) marked below:

1. ☐ fee calculation sheet
2. ☒ separate signed power of attorney 2 (two) forms
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: accompanying letter

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: ENGLISH

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

GERVASI Gemma

Milan, 23rd February 1999

For receiving Office use only

1. Date of actual receipt of the purported international application: (24.02.99) 24 FEB 1999
2. Drawings: ☒ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

NOTARBARTOLO & GERVASI S.P.A.
Corso di Porta Vittoria, 9
I-20122 Milano
ITALIE

NOTARBARTOLO & GERVASI MILANO
RECEIVED
- 1 GIU. 2000

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	30.05.2000
Applicant's or agent's file reference 1845PTWO.	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/01191	24/02/1999	25/02/1998

Applicant
FIDIA ADVANCED BIOPOLYMERS S.R.L.et.al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Hardy Magliano, N Tel.+49 89 2399-8151

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
1845PTWO.		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/01191	24/02/1999	25/02/1998
International Patent Classification (IPC) or national classification and IPC		
C08G18/83		
Applicant		
FIDIA ADVANCED BIOPOLYMERS S.R.L.et.al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21/09/1999	30.05.2000
Name and mailing address of the international preliminary examining authority:	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Hoffmann, K Telephone No. +49 89 2399 8419

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/01191

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-19 as originally filed

Claims, No.:

1-19 as originally filed

Drawings, sheets:

1-4 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-19
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-19
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-19
	No:	Claims	

2. Citations and explanations

see separate sheet

ad item V:

1. Reference is made to the following documents:
   D1 US 4 487 865 A
   D2 WO 95 25751 A.

2. The application refers to a polyurethane to which sulphated derivatives of hyaluronic acid are covalently bound. The products can be used as haemocompatible materials.

3. The subject-matter of claims 1 to 19 is novel because none of the prior art document discloses said sulphated derivatives of hyaluronic acid which are covalently bound to a polyurethane.

4. Document D2 is regarded as representing the closest prior art. It discloses sulphated derivatives of hyaluronic acid (claim 8) and their use in the wide variety of applications known in the art for hyaluronic acid-based biomaterials (page 7, lines 14-17), either alone or in **association** with other chemical polymers such as polyurethanes (page 28, lines 19-20).

   The claimed subject-matter differs from D2 in that the sulphated derivatives of hyaluronic acid must be **covalently** bound to the polyurethane. The problem underlying the present application was the provision of novel polymers with a high degree of biocompatibility and haemocompatibility. It was not obvious from D2 that the sulphated derivatives could keep their desirable anticoagulant properties when covalently bound to a polyurethane. Thus the skilled person could get no incentive to prepare the products according to present claim 1.

   Document D1 refers in example 3 to a polyurethane article which contains the sodium salt of hyaluronic acid covalently bound to polyurethane. The description of D1 as well as examples 4 and 5 and the claims refer to **acrylic** polymeric articles modified with hyaluronic acid. These articles have improved biocompatibilty. D1, however, is silent with respect to any properties of the polyurethane articles of example 3. The skilled person thus had no incentive to combine the teaching of example 3 of D1 with that of D2 in order to provide the

novel polymers according to claim 1.

5. It thus would appear that the present application complies with the requirements of Art. 33 PCT.